REMARKS

Applicant respectfully requests reconsideration of the above-identified patent application in view of the amendment above and the remarks below.

No claims have been canceled herein. Claims 1 and 13 have been amended herein. New claim 14 has been added herein. Therefore, claims 1-14 are pending and are under active consideration.

Claim 13 stands rejected under 35 U.S.C. 112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." In support of the rejection, the Patent Office states the following:

> Claim 13 is indefinite for the recitation of the phrase, "the method according to one of the preceding claims," because it is unclear to which of the above claims, claim 13 is dependent on. For the purpose of prosecution, the phrase has been assumed to mean, "the method according to **any** of the preceding claims."

Claim 13 has been amended herein and no longer recites the language in question. Therefore, the subject rejection is moot and should be withdrawn.

Claims 1-3, 6-10, 12 and 13 stand rejected under 35 USC 103(a) "as being unpatentable over Gonzalgo et al. (WO 98/56952, published December 17, 1998) in view of Yurov et al. (Human Genetics, 1996, vol. 97, pages 390-398) and in light of Davis et al. (U.S. Patent No. 6,046,002, issued April 4, 2000, filed January 5, 1998)."

> Gonzalgo et al. disclose a method of fluorescently detecting the methylated cytosine in a genomic DNA sample, wherein the genomic DNA is first treated with a bisulfite (page 4, line 15; claim limitation 6), the DNA amplified by PCR or polymerase chain reaction, incorporating radioactively labeled dNTPs, such as dCTP and dGTP (page 7-8; claim limitation 7), amplicons separated via electrophoresis (page 5, line 24; claim limitation 3), and the

amplicons detected radioactively (page 4, lines 10-30) or fluorescently (page 8, lines 30-31; claim limitation 12). Gonzalgo et al. also disclose a method of detecting the methylated cytosine, wherein the amplicons are transferred onto a nylon membrane for dot-blot analysis (page 8, lines 34-35; claim limitation 2 and 10).

Gonzalgo et al., while employing radioactively labeled dNTPs in the amplification step, do not employ fluorescently labeled dNTP.

Gonzalgo et al. do not employ the differentially labeled fluorescently labeled NTPs comprising cy3 and cy5.

Yurov et al. disclose the use of multicolor fluorescent detection via use of cyanine dyes, more specifically cy3 and cy5.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the teachings of Gonzalgo et al. with the teachings and suggestions of Yurov et al. to arrive at the invention as claimed for the following reasons.

Although Gonzalgo et al. employ radioactively labeled dNTPs and not fluorescently labeled dNTPs, particularly cy3 and cy5 labeled dNTPs, Gonzalgo et al. acknowledges alternate ways of labeling nucleotides (*i.e.* - fluorescent labels; see page 8, lines 30-31).

In addition to this acknowledgment, Yurov et al. disclose an explicit benefit provided by the use of cy3 and cy5 over the traditional fluorescent labels:

"Cyanine dyes are also useful as fluorescent labels or biological macromolecules. Cyanine 3 dye provides significantly **brighter** fluorescence than any other fluorophore, including fluorescein..." (page 391, 1st column).

Yurov et al. also disclose the advantage of using cy3 and cy5 dyes for multicolor detection assays (page 391, 2nd column).

Additionally, it is an art-recognized advantage that the use of fluorescent labels are environmentally safer as well as more efficient.

Therefore, one of ordinary skill in the art at the time the invention was made would have been motivated to take the

suggestion of Gonzalgo et al. and the advantage offered by Yurov et al. as well as art-recognized advantage of using fluorescent labels over the radioactive labels to arrive at the claimed invention. Since the substitution of fluorescently labeled nucleotides have been well established in the art of nucleic acid amplification and detection as evidenced by Davis et al.:

"Amplified sequences can be labeled by, for example, incorporation of a labeled nucleotide (e.g., a fluorescent nucleotide such as Cy3-dUTP or Cy5-dUTP, or a radioactive nucleotide" (Davis et al., at column 19, lines 18-20)

one of ordinary skill in the art would have had a clear expectation of success at substituting the radioactive labeling with fluorescent labels provided by Yurov et al.

Applicants' arguments received on April 19, 2004 have been fully considered but they are not found persuasive for the reasons set provided below.

Applicants' argument regarding the use of fluorescent markers by Gonzalgo et al. being after the amplification is rendered moot in view of the present rejection.

Applicants' arguments drawn to the method of Gonzalgo et al. only permitting the analysis of a single CpG dinucleotide positions is not found persuasive as the instant method steps read on the method of Gonzalgo et al. in view of the obviousness reasoning set forth above.

Applicants' arguments drawn to the hybridization of the "amplificate" [*sic*] to an oligonucleotide is also not found persuasive because Yurov et al. as well as Davis et al. discusses the hybridization of the amplicons to an array of immobilized oligonucleotides (for example, *Davis et al.*, column 19, lines 28-30).

Therefore, the invention as claimed is obvious over the cited references. (Emphasis in original).

Applicant respectfully traverses the subject rejection. Claims 2-3, 6-10, 12 and 13 depend from claim 1. Claim 1, which has been amended herein, now recites "[a] method for the relative

quantification of the methylation of cytosine bases in DNA samples, said method comprising the steps of:

a) chemically reacting a genomic DNA sample with a reagent, wherein 5-methylcytosine and cytosine react differently and these thus show a different base pairing behavior in the DNA duplex after the reaction;

b) then, amplifying the chemically reacted DNA sample, said amplifying step comprising the use of a fluorescently labeled dCTP or dGTP derivative to yield amplified products;

c) then, spatially separating the amplified products from each other; and

d) then, quantitatively measuring the fluorescence of the separated amplified products."

Claim 1 is distinguishable over Gonzalgo et al. for at least the reason that Gonzalgo et al. fails to teach or to suggest the claimed sequence of steps comprising (i) chemically reacting the genomic DNA sample; (ii) then, amplifying the treated genomic DNA sample while incorporating a fluorescently labeled dCTP or dGTP thereinto; (iii) then, spatially separating the amplified products; and (iv) then, quantitatively measuring the fluorescence of the separated amplified products.

Instead, as noted previously, Gonzalgo et al. relates to a markedly different method than that presently claimed. More specifically, Gonzalgo et al. teaches a method that involves (a) obtaining genomic DNA from a DNA sample to be assayed; (b) reacting the genomic DNA with sodium bisulfite to convert unmethylated cytosine residues to uracil residues while leaving any 5-methylcytosine residues unchanged to provide primers specific for the bisulfite-converted genomic sample for top strand or bottom strand methylation analysis; (c) performing a PCR amplification procedure using the top strand or bottom strand specific primers; (d) isolating the PCR amplification

products; (e) performing a primer extension reaction using Ms-SNuPE primers, [^{32}P]dNTPs and *Taq* polymerase, wherein the Ms-SNuPE primers comprise a from about a 15 mer to about a 22 mer length primer that terminates immediately 5' of a single nucleotide to be assayed; and (f) determining the relative amount of allelic expression of CpG methylated sites by measuring the incorporation of different ^{32}P-labeled dNTPs.

One distinction between the claimed method and the method of Gonzalgo et al. is that the Gonzalgo method does not involve amplification of the genomic DNA sample using fluorescently labeled dinucleotides. Instead, in Gonzalgo et al., (i) a radioactive marker is used instead of fluorescently labeled dinucleotides and (ii) the radioactive marker is incorporated into the oligonucleotide only **after** amplification of the genomic DNA sample has already been performed. By contrast, in the claimed method, the incorporation of the fluorescently labeled dinucleotide takes place **during** amplification, the claimed method not even reciting a primer extension step.

Applicant also wishes to note that whereas the Gonzalgo method only permits the analysis of single CpG dinucleotide positions, the claimed method permits the quantification of the level of methylation within complete nucleic acid segments.

The Patent Office has attempted to cure the above deficiencies of Gonzalgo et al. by combining Gonzalgo et al. with Yurov et al. and Davis et al. Yurov et al. and Davis et al. are apparently being relied upon by the Patent Office to support replacing the radioactive markers of Gonzalgo et al. with fluorescently labeled dNTPs. However, even assuming *arguendo* that there is a basis for making such a substitution (an assumption Applicant does not concede), Applicant respectfully submits that Yurov et al. and Davis et al. provide no basis for modifying Gonzalgo et al. so that the labeling step takes place during the amplification step, as opposed to during the primer

extension step. Modifying Gonzalgo et al. to render completely unnecessary its primer extension step would constitute a complete overhaul of the Gonzalgo method and would not have been suggested to a person of ordinary skill in the art in view of the other references.

Therefore, for at least the above reasons, claim 1 is patentable over Gonzalgo et al. in view of Yurov et al. and in light of Davis et al.

The claims dependent from claim 1 recite additional features and are further patentable over Gonzalgo et al. in view of Yurov et al. and in light of Davis et al. for at least the reasons of record.

New claim 14 is patentable over the applied combination of references for at least the same reasons discussed above in connection with claim 1. In addition, Applicant notes that claim 14 uses the transition phrase "consisting of."

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

Claims 1-13 stand provisionally rejected "under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-29 of copending Application No. 10/220,090."

Applicant respectfully requests that the foregoing provisional rejection be held in abeyance until after the Patent Office has allowed the '090 application.

Claims 1-13 stand provisionally rejected "under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-26 of copending Application No. 10/220,896."

Applicant respectfully requests that the foregoing provisional rejection be held in abeyance until after the Patent Office has allowed the '896 application.

In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

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Dated: November 22, 2004

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Fee Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on November 22, 2004

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